THE GROWTH OF LARVÆ OF *AMBYSTOMA MACULATUM* UNDER NATURAL CONDITIONS

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Introduction

Attempts to describe the increment in length and weight during the larval history of amphibians are confronted with either of two difficulties. If the animals are raised in the laboratory at a constant temperature, the normal or optimum food conditions cannot be duplicated readily; if animals are collected periodically from their natural habitat, the conditions of life there are so variable as to produce data difficult to describe. Accordingly the literature is neither extensive nor consistent.

Davenport (1897, 1899) presented a few data upon the weight increment of larvæ of "the common frog" under laboratory conditions. Three stages of growth are recognized; first a period of slow growth accompanied by abundant cell division, then a period of rapid growth due to imbibed water, and finally a period of equally rapid growth in which the increment is due to increase both in organic substance and water.

Schaper (1902) has provided data still more complete on the larval growth of *Rana esculenta* under laboratory conditions. The daily growth in weight and volume of these tadpoles is slight at first, gradually becoming greater and attaining a maximum value at about eighty days. During the following week these values fall to about half as the animal undergoes metamorphosis. During the first fourteen days of development the organic matter and ash remain constant (13 mgm. and 1 mgm. respectively). Weight increment during this period is due solely to imbibition of water. The percentage of solid then increases until maximum size is attained; during metamorphosis this percentage is further increased. Schaper's data on length increase cannot be easily interpreted, probably because too few specimens were considered.

Robertson (1923) apparently unaware of Schaper's work has attempted with indifferent success to convert Davenport's data into a mathematical expression. He believed that the weight increment in the frog tadpole could be expressed by a single symmetrical sigmoid curve.

Studies on salamanders have shown variable rates of growth. When the length-age data of Eycleshymer and Wilson (1910) on *Necturus* under laboratory conditions and those of Bishop (1926) dealing with the animal under natural conditions are plotted it may be seen that during the first three (or four) months of development (except for a period before the embryonic axis is straight) the length increases at a constant rate.[1] The nearly uniform yearly growth to the period of sexual maturity, which Bishop records, suggests that aside from thermal variations the rate of linear growth may be constant from year to year.

Wilder (1924), on the other hand, has shown that under natural conditions the rate of linear growth varies at different times during the larval history in *Spelerpes bislineata*. Although she disregarded the embryonic development, subsequent growth stages are recorded. The post-embryonic stage, until the yolk is absorbed, is the period of most rapid growth. The typical larval period during the fall and winter of the first year is a time of slow growth. The latter part of this period during the spring of the second year is characterized by rapid growth. The premetamorphic stage during the fall and winter of the second year involves a period of slow growth, then a period of fluctuating growth. The metamorphic stage in the summer of the third year is a period in which the catabolic changes are more pronounced than the anabolic.

It must be noted that *Spelerpes* does not become terrestrial until it has spent two years of its life in an aquatic habitat; *Necturus* is permanently aquatic. It seems likely that a more typical method of growth would be found in salamanders which have an aquatic stage lasting for only a single season. The increase in length of *Ambystoma* has been studied by two observers. Uhlenhuth (1919), who has studied *A. opacum* under constant conditions, stated that the rate of growth seems to be proportional to the velocity of metamorphosis (rate of growth $\times$ age at metamorphosis $=$ constant). He does not, however, describe the growth rate of various periods of development.

Patch (1927) has described the length increase in three groups of *Ambystoma* as consisting of two sigmoid curves, one embryonic and the other larval. The point of junction of these two curves is 15.61 mm. in *A. maculatum,* 14.07 mm. in *A. tigrinum,* and 11.96 mm. in the axylotl.

In order for curves of length increase to represent a fundamental

[1] Actually, Bishop's data shows a slight variation from the constant growth rate over a period of two weeks in July. The climatological records of the U. S. Weather Bureau for the Saegertown, Pa. region give a rise in temperature during this period which undoubtedly accounts for the fluctuation.

phase of growth, the relation between weight and length for successive stages must be constant, as Miss Patch has assumed. In view, however, of the marked changes in body form during the embryonic life of *Ambystoma* it seems unlikely that the " indices of build " are uniform. It is necessary to have data on both length and weight, at least to the point in development where the body form assumes nearly constant larval proportions, in order to correctly appreciate the body increment.

Material and Methods

During the spring of 1928, the author located a salamander pond sufficiently well populated with spawning *Ambystoma maculatum* to indicate that eggs and larval specimens could be obtained throughout the season. The present study involving about 1700 specimens is the outcome of two years of systematic collection from that habitat.

Delhi Pond is a shallow, sheltered, leafy-bottomed forest pond in the environs of Ann Arbor. It has a maximum area of one-twelfth acre and a maximum depth of about four feet; it is ordinarily a permanent pond but became dry during the season of 1929. In addition to *A. maculatum,* the usual invertebrate fauna, *A. tigrinum* and *Rana cantabrigensis* were present.[2]

From the time when eggs were first observed until the salamanders metamorphosed, periodic random collections were made, the intervals between successive collections being seldom more than a week. Thirty to forty specimens formed a sample although occasional collections, particularly during the early stages, amounted to more than a hundred specimens. Specimens were collected by dredging the bottom of the pond with a hand net formed of a yard of wire netting stretched between two poles. All parts of the pond were sampled so that the collection is quite representative. The maximum-minimum temperatures of the pond were recorded at the time of collection.

The specimens were brought to the laboratory alive, anesthetized and measured, mutilated specimens having been discarded. During the non-motile stages anesthesia was not required. The eggs and embryos up to the period of hatching were placed for measurement upon the stage of a binocular microscope fitted with a camera lucida.

[2] Although B. G. Smith (1911) stated that *Ambystoma tigrinum* and *A. maculatum* are not found in the same habitat in the Ann Arbor region, the author found both salamanders in abundance in the present location. The two populations are more or less independent of one another and it is not evident that the larger salamander through its predatory activities seriously affects the *A. maculatum* population. The eggs and larvæ of the two species could not be confused easily since the egg clutches, the time of hatching, the size and appearance of embryos and larvæ are distinctly different.

The image of the embryo could thus be superimposed on a properly calibrated scale. The larger, later specimens were measured by means of drafting calipers and millimeter rule. In the blastula and non-motile stages the maximum diameter or length irrespective of the curvature of the body axis in relation to the yolk mass was recorded. The length taken in later stages when the axis was linear was the maximum length.

The average weight at different developmental stages was also determined: The anesthetized salamanders were placed in a tared crucible and weighed after the excess water had been absorbed by pipette and filter paper. They were then dehydrated for several days in a drying oven at 95–97° C. and the dry weight determined. Following this the sample was incinerated over a Meeker burner for two to ten hours and the ash weighed.

Results

Changes in Weight to the Period of Metamorphosis.—During the year 1928, the first eggs were found on April 3. On August 18, many specimens had begun to metamorphose at a weight of about 1200 mgm. A week later there were relatively few specimens in the pond. During the year 1929 the first eggs were collected on March 27, and on August 14, a number of specimens, at approximately 800 mgm. had metamorphosed. When the average weight of a sample of salamander eggs, embryos or larvæ is plotted against the age, as in Figs. 1 and 2, the rate of growth may be expressed as a curve. The weight increment was slow at first, gradually increasing to the middle of June when the rate of increase became more and more rapid. By the middle of July the rate of growth was at its maximum. In the first week of August the growth rate was markedly reduced. Finally growth became negligible and metamorphosis occurred. The weight increase may be thus described as a single sigmoid curve. Under natural conditions the first stages of growth were considerably prolonged, due to the low water temperatures of spring. During the larval and premetamorphic stages the temperatures are more nearly the same. The terminal period of growth is very brief, so short in fact, that the curve of *Ambystoma* increment shows a marked variation from the curves of autocatalytic growth in other animals. During both years the same general type of sigmoid curve is demonstrated, although the actual weight values are considerably different.

Linear Increase to the Time of Metamorphosis.—The curve formed by plotting length against age shows a slight deviation during the first four or five weeks of development. From this point on the curve is sigmoid. Growth increment is gradually increased to a period within three to four weeks of metamorphosis when the rate of increase is considerably lowered, and finally becomes negligible. The deviation during the early stages of development, which Miss Patch has interpreted as a distinct period of growth, involving the typical sigmoid growth rates, is due to the form changes of the embryo. The embryonic axis from the neurula to the early limb bud stages is curved around the

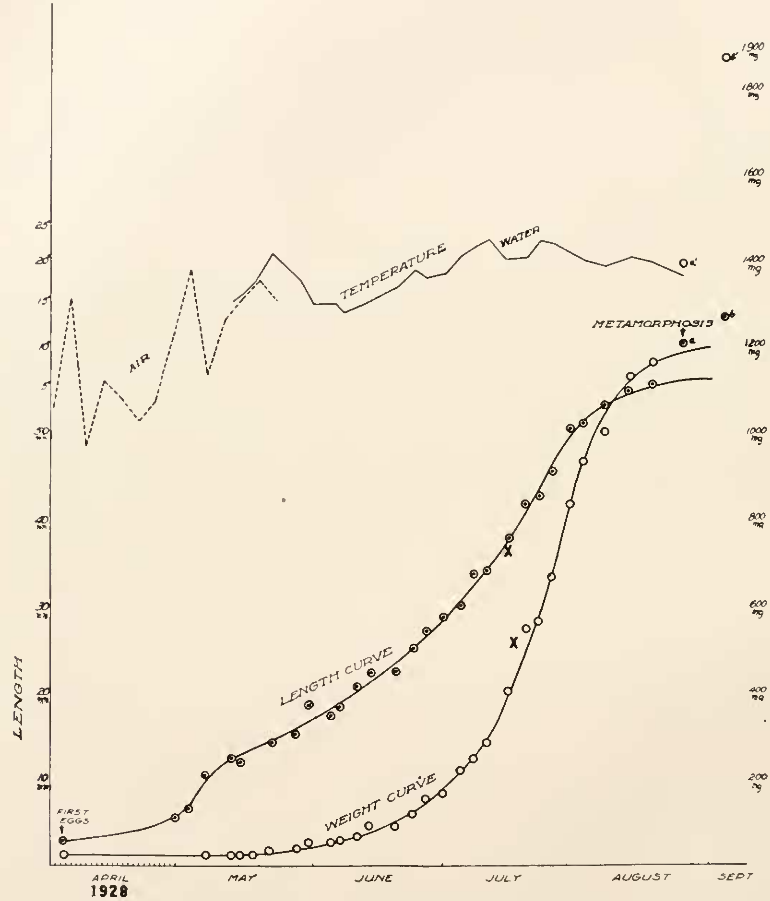

FIG. 1. A curve showing the relation between average length and weight of larval specimens of *A. maculatum* at various ages. Data of 1928.

yolk mass. Increase in length of this axis during the early growth stages does not result in equivalent increase in the total length of the embryo. It is not until the embryonic axis becomes straightened that the total length shows marked increase.

That the departure from a single sigmoid curve during the early development is not a distinct phase of growth may be demonstrated. When an "index of build" (Length ^{3}/Weight) is computed (Table I), it is clearly indicated that length and weight are not directly associated during the early stages. This index varies constantly to a period shortly before hatching when the embryonic axis becomes linear. It is fairly constant, however, for the free living larval stages.

A group of experiments carried under approximately constant temperature conditions gives indication that the degree of curvature may vary under environmental circumstances. Four groups of salamanders

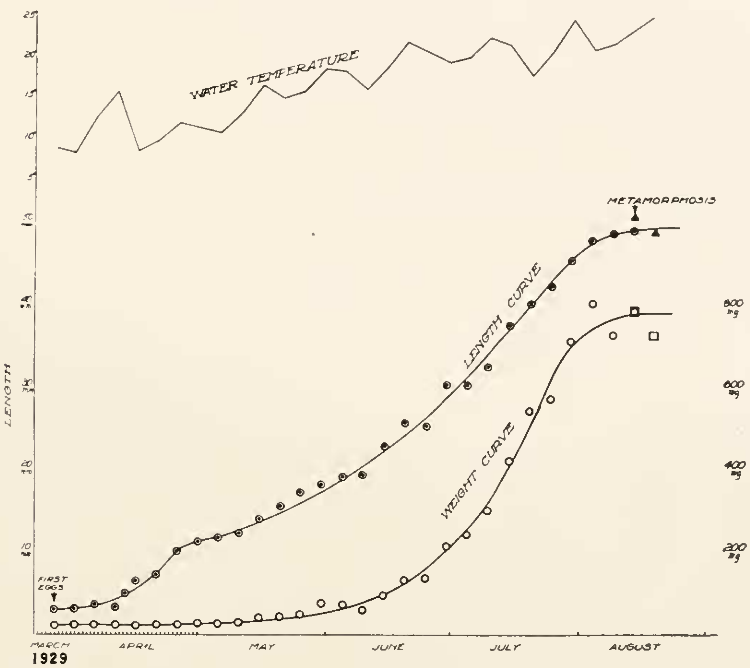

FIG. 2. Curve of growth showing weight and linear increment. Data of 1929.

at the neurula stage were placed at approximately constant temperatures of 4° C. ± 1, 13° C. ± 1, 19° C. ± 1 and 27° C. ± 1. When the four groups of length-age data acquired from these animals were plotted in such a way as to rule out the change in growth rate due to temperature, that is, when all the data, after allowance is made for appropriate thermal coefficients of growth, are plotted as though the animals were raised at 19° C., the shape of the curve is not the same for each group. The linear increase in the 4° sample was practically

Table I

Giving the average lengths, weights and analyses at different stages of embryo and larval A. maculatum. Data of 1929

Date	Mean Length	Average Wet Weight	$\frac{\text{Length}^3}{\text{Weight}}$	Water	Dry Weight	Ash	Water	Dry Weight	Ash	Stage
	mm.	*mgm.*		*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*	*per cent*	
Mar. 27.....	2.21 ± .08	6.16	1.8							7–9 (Harrison)
Apr. 1.....	2.37 ± .11	7.32	1.8	4.98	2.34	.097	68.03	31.97	1.32	10–13
Apr. 6.....	2.75 ± .31	6.89	3.0	4.73	2.17	.070	68.58	31.42	1.01	15–20
Apr. 11.....	4.55 ± .99	8.43	11.2	5.70	2.73	.081	67.62	32.38	.96	26–31
Apr. 16.....	5.81 ± .14	6.98	28.1	4.51	2.48	.095	64.54	35.46	1.36	33±
Apr. 21.....	6.56 ± .15	7.85	35.9	5.53	2.33		70.38	29.62		36±
Apr. 26.....	9.45 ± .90	9.50	88.8	6.93	2.57		72.94	27.06		34–39
May 1.....	10.51 ± .77	9.78	118.6	7.54	2.24		77.08	22.92		39–41
May 6.....	11.09 ± .52	11.28	120.8	8.82	2.46		78.19	21.81		
May 11.....	11.76 ± .61	11.52	141.1	9.52	2.00	.20	82.63	17.37	1.74	42–43 Hatching
May 16.....	13.41 ± .61	25.3	95.2	22.87	2.43	.30	90.38	9.62	1.18	42–45
May 21.....	15.01 ± .75	27.34	123.6	25.89	2.45	.29	94.68	5.32	1.04	45 (yolk nearly gone)
May 26.....	16.75 ± 1.00	36.38	129.1	33.43	2.96	.38	91.86	8.14	1.04	46+ (eating?)
May 31.....	17.61 ± 1.33	63.64	85.8	58.66	4.98	.62	92.17	6.42	.97	
June 5.....	18.52 ± 1.64	58.42	108.7	53.47	4.95	.70	91.52	8.47	1.20	
June 10.....	18.65 ± 1.69	44.96	144.4	40.67	4.29	.45	90.45	9.54	1.00	
June 15.....	22.2 ± 2.33	83.56	130.8	75.68	7.88	.92	90.56	9.43	1.10	
June 20.....	25.18 ± 2.19	119.55	133.8			1.50			1.26	
June 25.....	24.7 ± 2.85	121.40	124.1	109.35	12.05	1.45	90.08	9.93	1.20	
June 30.....	29.93 ± 2.48	204.37	132.1	184.13	20.24	2.53	90.09	9.91	1.24	
July 5.....	29.8 ± 2.50	235.68	114.8	211.24	24.44	2.96	89.63	10.37	1.25	
July 10.....	32.0 ± 2.89	289.41	113.3	255.48	33.93	3.78	88.28	11.72	1.30	
July 15.....	37.21 ± 2.32	412.54	124.5	366.97	45.57	5.91	88.95	11.05	1.43	
July 20.....	39.78 ± 3.02	538.21	117.1	477.16	61.05	7.61	88.66	11.34	1.41	
July 25.....	41.94 ± 2.94	565.00	130.1	496.00	69.00	8.07	87.79	12.21	1.43	
July 30.....	45.10 ± 3.71	709.45	128.4	618.03	91.43	11.10	87.11	12.89	1.56	
Aug. 4.....	47.70 ± 4.17	798.68	135.8	694.13	104.55	11.55	86.91	13.09	1.45	
Aug. 9.....	48.50 ± 2.68	722.50	157.9	624.30	98.20	11.62	86.41	13.59	1.61	
Aug. 14.....	48.92 ± 2.36	785.63	148.8	673.77	111.86	13.30	85.76	14.24	1.69	
	50.50	784.50	164.1	667.25	117.25	15.25	85.05	14.95	1.94	Terrestrial
Aug. 19.....	48.70 ± 2.56	717.10	161.1	590.60	126.50	16.90	82.35	17.65	2.36	"

a straight line growth. Some deviation from this type of growth was found in the 13° data, more in the 19° data and still more deviation in the 27° data. It is very unlikely that the weight differs in these groups. High and low temperatures apparently affect the efficiency of the cardiovascular mechanism so that atypical individuals are eventually produced. Under high temperature conditions the embryo folds around the yolk, develops rapidly and eventually straightens its axis; under the low temperature yolk is not readily utilized and the head and tail buds from the time of their formation extend away from the yolk mass rather than lie close to it.

In all the data provided by salamander collections under natural conditions, it may be noted that the "probable error of length" has a more or less constant ratio to the average length determinations of the various stages. The population may thus be considered to be fairly homogeneous concerning the individual growth rates.

Relation of Weight and Length.—Aside from the deviation between length and weight in the early embryonic stage, due to embryonic foldings, there are certain other fluctuations. Occasional samples from two other ponds compared with 1928 curves indicated that for a certain weight, there were considerable variations in length. In the period before the animals began to feed there was little difference in these values but later the differences were marked. When the 1929 curves are superimposed on the 1928 curves this relation is brought out clearly. The curves of linear growth and the weight curves practically coincide to the point *X* of Fig. 1. From this point to the period of metamorphosis the variation is great. A higher average "index of build" is found for the data of the first year as compared with that of 1929.

Time of Metamorphosis.—It seems quite probable that the actual time of metamorphosis under natural conditions is associated with the conditions of life in the pond. In August, 1929, the pond under consideration became dry. The growth weight as evidenced by the curve showed a marked slowing down toward the end of July, while in the previous year, under more favorable conditions, this was not evident until the first week in August. During this first year, in fact, there were specimens in the pond for at least two weeks after most of the salamanders had metamorphosed.[3] This laggard group was formed of

[3] R. G. Harrison (Correlation in the development and growth of the eye, etc. *Arch. f. Entw.-Mech.*, Bd. 120, 1929) has figured three curves for the post-embryonic linear growth of *A. maculatum* larvæ under laboratory conditions. These curves, which are sigmoid, indicate that the rate of growth is accelerated with increased feeding and, in contrast to the present data, that the length at metamorphosis is constant (47–50 mm.) under different feeding conditions. A similar curve is given by L. S. Stone (Heteroplastic transplantation of eyes between the larvæ of two species of *Amblystoma. Jour. Exp. Zoöl.*, Vol. 55, 1930).

relatively large specimens (Fig. 1, *a*, *a'*, *b*, *b'*). It seems quite probable that these specimens had not yet entered the third period of growth, *i.e.* the terminal period of slow growth. During the second year, when the pond became dry, two records, one before the pond became dry and the other immediately afterward, are available on the length and weight of recently metamorphosed specimens. In the first of these both values are higher than in the second. The second group was undoubtedly " forced " by the drying of the pond to metamorphose before reaching the stage at which the first group metamorphosed.

Allee (1911), who has studied the seasonal succession of pond fauna, has indicated that there is a periodic change in numbers of species and individuals found in forest ponds. There is an increase in numbers of species which is slow during the spring months and rapid in early summer, less marked in July and in late August the number falls to the spring value. There seems to be a correlation between the period of highest productivity of the pond as reported by Allee and the period of rapid growth of the salamanders recorded here.

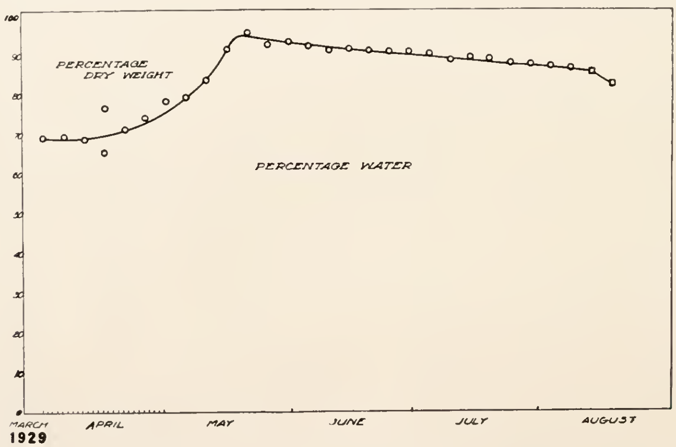

Fig. 3. Graph showing the relative percentage of water and organic substance in larval salamanders of different ages. Data of 1929.

Relations of Water, Solids and Ash to Growth.—The eggs shortly after they were laid had a weight of 7.32 mgm. consisting of 4.98 mgm. of water and 2.34 mgm. of solid, of which .097 mgm. was ash. During the embryonic period the dry weight was fairly constant. Actual increase was associated with increase in inorganic matter and water (Table I). The ash percentage, however, was practically constant while the water increased in this period from 68 to 94 per cent. After the animals began to eat, the dry weight increased considerably so that the percentage of water decreased. To the period of metamorphosis there was a gradual increase of inorganic matter from 1 to 2 per cent. Water per cent decreased from 94 to 85 per cent and the percentage dry weight increased from 6 per cent to 15 per cent. This relationship is brought out in Fig. 3. Until the animals began to feed, growth was purely a process of hydration; afterwards it was due both to imbibition of water and to increase in organic and inorganic materials. These findings are in accord with the work of Davenport and Schaper on the Anura. Recently metamorphosed specimens showed still further decrease in the percentage of water content. Data on the percentage of water in older metamorphosed specimens (Table II) show that this early decrease may be later compensated. The proportion of dry weight, ash and water, however, seems to be variable for the land forms. Two specimens from an indoor aquarium in December showed a decrease in water content to 80 per cent body weight and an increase in inorganic matter to 4 per cent.

Table II

Showing the relative content of water, solids and ash in terrestrial stages of A. maculatum.

Length	Wet Weight	$\frac{\text{Length}^3}{\text{Weight}}$	Water	Dry Weight	Ash	Water	Dry Weight	Ash
mm.	*grams*		*grams*	*grams*	*grams*	*per cent*	*per cent*	*per cent*
September 1929								
50.5	.603	214	.491	.112	.011	81.51	18.49	1.77
71	1.659	218	1.412	.247	.031	85.12	14.88	1.86
76	2.504	177	2.206	.298	.032	88.10	11.90	1.27
82	4.616	120	4.063	.553	.084	88.02	11.98	1.82
88	4.709	146	4.012	.697	.101	85.10	14.80	2.14
93	5.602	144	4.955	.647	.102	88.45	11.55	1.82
104	5.875	193	4.939	.936	.124	84.07	15.93	2.11
December 1928								
136	9.432	269	7.848	1.584	.427	83.21	16.79	4.53
138	8.792	301	7.044	1.748	.366	80.12	19.88	4.16

Summary

1. Growth in weight of embryonic and larval *Ambystoma maculatum* from the time that eggs are deposited to the period of metamorphosis may be expressed as a single sigmoid curve.

2. The length curve, except for a short period before hatching when the embryonic axis is curved, is likewise sigmoid.

3. The *Ambystoma* population of a pond is quite homogeneous, the specimens metamorphosing at approximately the same time.

4. Under natural conditions the relation between weight and length from year to year seems to be constant during the stages before feeding. Later the relationships are variable because of feeding differences.

5. Growth to the time of food ingestion is associated with imbibition of water. Later growth to the time of emergence of the salamanders is correlated with a process in which the percentage of water content decreases. During this period the inorganic constituents gradually increase.

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